JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1985, p. 245–249
0095-1137/85/080245-05$02.00/0

Vol. 22, No. 2

Aggregation of Platelets by *Fusobacterium necrophorum*

LAWRENCE J. FORRESTER,[1] BENEDICT J. CAMPBELL,[1] JOHN N. BERG,[2] AND JAMES T. BARRETT[3]*

Departments of Biochemistry,[1] Veterinary Microbiology,[2] and Microbiology,[3] University of Missouri, Columbia, Missouri 65212

Received 25 February 1985/Accepted 30 April 1985

Broth cultures and washed cells of 13 of 24 bovine isolates of *Fusobacterium necrophorum* aggregated human platelets in platelet-rich plasma. The cell-free culture fluid was inactive. Bacteria stored at 4°C in saline remained active for at least 3 months, but they did not release activity into the storage solution. Aggregation typically began within 1 min after the addition of 10^3 bacteria to 10^3 platelets and was complete within 5.5 min. Assays for cytosolic lactic dehydrogenase revealed that platelet lysis did not occur. The release of [^{14}C]serotonin from platelets preincubated with this amine accompanied aggregation, indicating that this was a typical aggregation-degranulation reaction. Platelet aggregation was inhibited by EDTA (88% at 2.0 mM), aspirin (75% inhibition at 1.0 mM), and quinacrine (80% inhibition at 0.25 mM). Thus the reaction was an ion-dependent, cyclooxygenase-sensitive event. Gel-filtered platelets were less sensitive to aggregation than were platelets in plasma, but this sensitivity was fully restored by the addition of plasma and partially restored with fibrinogen. Biotyping of the cultures revealed that none of the avirulent, B-type strains of *F. necrophorum* could aggregate platelets, whereas 13 of 16 virulent A type strains were positive. These results suggest that platelet aggregation by *F. necrophorum* is related to the virulence of this organism.

The ability of fusobacteria to form abscesses in deep-seated organs and soft tissues is well established, but the virulence factors used by these organisms in this process remain virtually unknown (15). A galactose-sensitive adhesin (25) of *Fusobacterium nucleatum*, a known associate of human periodontal disease, enables it to establish intimate contact with oral epithelial cells (10) and with other oral bacteria involved in gingivitis and dental plaque formation (8). Indeed, *F. nucleatum* has been described as coaggregating with a broader range of oral bacteria than do other buccal inhabitants (P. E. Kolenbrander, R. N. Anderson, and L. V. Holdeman, Abstr. Annu. Meet. Am. Soc. Microbiol., 1985, B52, p. 26). This may permit a synergistic relationship to develop with other anaerobes, notably *Bacteroides* species and gram-positive cocci (14) that would enhance the opportunity for disease. Although several hydrolytic enzymes are elaborated by *Bacteroides* species (39), the fusobacteria are comparatively inert in this regard. Activation of the alternate complement pathway by fusobacterial lipopolysaccharide has been described as a factor in inflammatory periodontal disease (27). The production of large quantities of a low-molecular-weight leukotoxin by virulent type A strains of *Fusobacterium necrophorum*, in contrast to the low quantities produced by the avirulent B strains is one of the few definitive descriptions of a virulence factor in this genus (34). Our study reported here indicates that platelet aggregation may also be a virulence property of *F. necrophorum*.

Platelet aggregation has been suggested as a virulence property of other microorganisms that cause thrombocytopenia, disseminated intravascular coagulation, fibrin deposition, and other coagulative effects (33). The cell membrane of the platelet is sensitive to many stimuli that provoke aggregation and degranulation. The binding of immunoglobulin G to the platelet Fc receptor renders the platelet susceptible to aggregation by strains of *Staphylococcus aureus* that contain protein A in their cell wall (13). The dependence of platelet aggregation by *Streptococcus pyogenes* on the presence of fibrinogen (20) and the evidence that both streptococcal M and T proteins function as fibrinogen receptors (19, 35) on this organism are useful in explaining the mechanism of the platelet reaction in this instance. Activation of platelets by the yeast phase of *Histoplasma capsulatum* requires fibrinogen and immunoglobulin G (7). The nonlytic activation of platelets by *Listeria monocytogenes* requires an unidentified, heat-labile serum factor that is not complement (6).

Due to the presence of receptors for complement component C3b on platelets, agents that activate the complement system, including microbial lipopolysaccharide (26), tuberculin (32), *Aspergillus* antigens (36), streptococcal teichoic acid (2), peptidoglycan (12, 31), and *Candida* mannans (22, 37), all cause platelet aggregation which is often followed by platelet lysis. A number of microbial enzymes, e.g., the lecithinase of *Clostridium perfringens* (*C. welchii*), the alpha toxin of *Staphylococcus aureus*, streptolysins S and O (3), and pneumolysin (18), lyse platelets by dissolving their cell membranes and thus forcing platelet degranulation and an interruption of the blood clotting system.

MATERIALS AND METHODS

Bacterial cultures. All strains of *F. necrophorum* used in this study were isolated from cattle and identified by the standard microbiological procedures applied to gram-negative anaerobes. The biotypes of the cultures were determined by the procedures of Fievez (9). Of the 24 strains, 16 were of the virulent biotype A, 7 were of the avirulent biotype B, and 1 strain was of the mixed biotype AB. Four strains of *Bacteroides fragilis* and one strain each of *Bacteroides melaninogenicus* and *Bacteroides thetaiotaomicron* from the culture collection of the Department of Veterinary Microbiology, School of Veterinary Medicine, University of Missouri-Columbia, were included. The organisms were grown for 24 h in broth incubated at 37°C in an anaerobic chamber. The cells were collected by centrifugation, washed three times in sterile saline, and suspended in saline. Nephelometric calibration against viable cell counts was used to establish the cell density.

* Corresponding author.

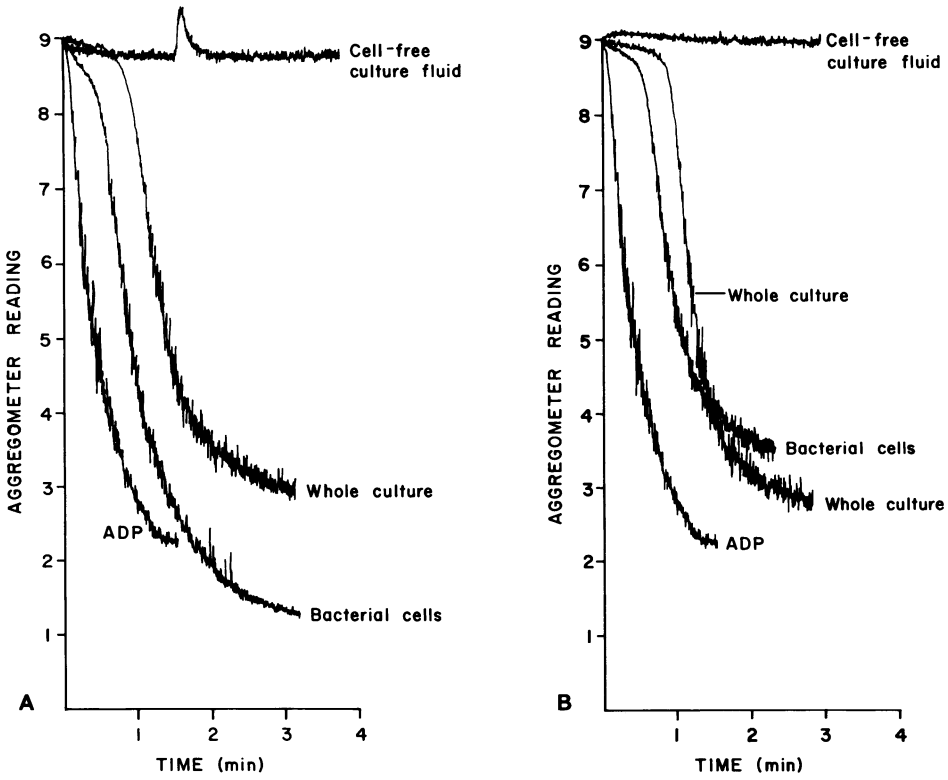

FIG. 1. Panels A and B display the platelet aggregation curves of whole cultures, washed bacterial cells, and the cell-free culture fluids of *F. necrophorum* strains 5018 and 3080, respectively. The response to ADP represents a positive control. The negative control responses to uninoculated culture media and saline are not presented since they were essentially superimposable on the cell-free culture fluid recordings.

Chemicals. [2-^{14}C]serotonin (side chain 2-^{14}C, 58 mCi/mmol) was purchased from Amersham Corp., Arlington Heights, Ill., and stored as a 100 μM solution in 0.5 M NaCl. All inhibitors and the reagents for the lactic acid dehydrogenase assay were purchased from the Sigma Chemical Co., St. Louis, Mo.

Platelets. Human blood from donors not on medication of any kind was the source of platelets. Nine volumes of blood were drawn into one volume of 3.8% sodium citrate solution in a plastic syringe. This mixture was transferred to plastic tubes and centrifuged at 150 × *g* for 10 min, after which the supernatant fluid was collected into plastic tubes and used as platelet-rich plasma (PRP). PRP was held at room temperature and used within 4 h.

Gel-filtered platelets (GFP) were prepared by passing 2.0-ml portions of the PRP preparation through a Sepharose 2B column. The column consisted of a 10-ml plastic pipette packed to a height of 20 cm. The gel-suspending and -eluting fluid consisted of Tyrode solution supplemented with 200 μg of apyrase per ml and containing 0.1% glucose and 0.35% bovine serum albumin.

Platelet numbers were determined and adjusted to 300,000/ml by the addition of platelet-poor plasma consisting of the supernatant fluid after centrifugation of PRP at 1,640 × *g* for 10 min (4, 5).

Platelet aggregation. A single-channel Chronolog aggregometer (model 300-1; Chrono-log Corp., Havertown, Pa.) attached to an Omniscribe recorder (model 5112-1; Houston Instruments, Austin, Tex.) was used to measure and record platelet aggregation (20). In each experiment, platelet-poor plasma was used to adjust the instrument to 100% transmission to represent 100% aggregation. A mixture of 450 μl of either PRP or GFP and 50 μl of the Sr^{2+} Tyrode buffer (21) was used to represent 0% transmission (aggregation).

In the actual experiments, 450 μl of PRP or GFP was added to the cuvette, which was placed in the aggregometer. After the suspension had warmed, 50 μl of the bacterial suspension was added, and the zero point was readjusted due to the density of the bacterial suspension. Platelet aggregation was followed for a minimum of 6 min. In a companion experiment, an addition of 50 μl of ADP to achieve a final concentration of 1.5 μM was used as a positive control to ensure suitability of the platelet preparation.

In the inhibition experiments, aspirin, quinacrine, dipyridamole, imipramine, or EDTA was preincubated with the PRP for 10 min at room temperature before being transferred to the 37°C environment in the aggregometer. When this temperature was achieved, the bacteria were added to initiate the experiment. Likewise, in the fibrinogen experiment, the GFP and fibrinogen were mixed and preincubated for 10 min at room temperature. Otherwise, the experiment was conducted as with the inhibitors.

Serotonin and lactic acid dehydrogenase release. Platelet aggregation and serotonin and lactic acid dehydrogenase release were measured over the same time period from a common platelet preparation. Platelets were labeled by incubation of PRP with 1 μM[^{14}C]serotonin for 30 min at room temperature before their use in the aggregation assay. After a specified time of incubation in the aggregometer, a 450-μl portion was removed and added to 75 μl of 933 mM formaldehyde containing 50 mM EDTA in a 1.5-ml centrifuge tube held in ice. The tubes were centrifuged in a

Beckman model B microfuge at approximately 12,000 × *g* for 2 min. Duplicate 200-μl samples were removed and added to 10 ml of Brays solution and subsequently analyzed in a Packard 460 CD liquid scintillation counter. Total radioactivity in the samples was determined from uncentrifuged controls. Release of radiolabeled serotonin was calculated from the formula: % release = *S* test − *S* control/*T* control − *S* control × 100, where *S* equals the radioactivity in the supernatant fluid and *T* equals the total radioactivity measured as disintegrations per minute (17). By altering the time allowed for aggregation and serotonin release by 0.5-min intervals, the comparative kinetics of these reactions could be determined.

Lactic acid dehydrogenase released from platelets during these experiments was determined by a standard method (2) after an aggregation time of 6 min. A 10% solution of Triton X-100 was used to lyse platelets to determine the total amount of intracellular lactic acid dehydrogenase.

RESULTS

Broth cultures of 4 strains of *B. fragilis*, 1 of *B. thetaiotaomicron*, 1 of *B. melaninogenicus*, and 24 of *F. necrophorum* were tested for their ability to aggregate platelets. Of the *Bacteroides* spp., only one culture of *B. fragilis* was active, in contrast to the finding that 13 of the 24 cultures of *F. necrophorum* aggregated platelets within 6 min. The kinetics of aggregation by *F. necrophorum* strains 3080 and 5018 are recorded in Fig. 1, reflecting the general aggregating ability of active strains of this species. Aggregation by the whole broth culture normally began within 1 to 2 min. Platelets mixed with washed bacterial cells began to aggregate about 30 s earlier. Aggregation was essentially complete within 2 min, although three less potent strains required 5.5 min to reach maximum aggregation. A total of 11 cultures were inactive. Although ADP was able to initiate platelet aggregation more quickly than bacteria, the bacteria aggregated the cells to the same extent as ADP (aggregometer readings between 1.5 and 3). Cell-free culture media were unable to activate platelets, and when used instead of saline for the suspension of washed bacteria, they displayed no inhibitory property. Saline, uninoculated media, and cell-free growth media were all totally inactive.

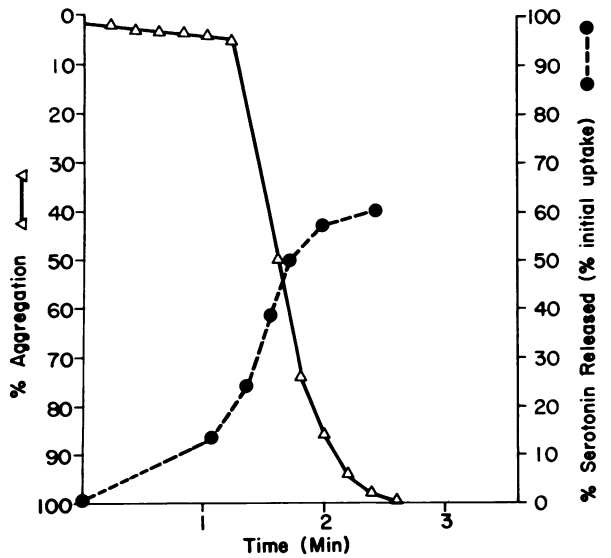

FIG. 2. This joint recording of platelet aggregation (△) and serotonin release (●) induced by *F. necrophorum* 5071 reveals that both functions were initiated and completed in approximately 2.5 min.

TABLE 1. Inhibitors of *F. necrophorum* 5071 platelet aggregation

Inhibitor	Concn (mM)	Time before aggregation onset (min)	% Aggregation at 6 min
None		1.5	100
Aspirin	1.0	2.0	25
Aspirin	2.5	2.5	12
Quinacrine	0.25	1.5	20
EDTA	2.0	2.5	12
Dipyridamole	0.5	2.0	30
Imipramine	0.01	2.5	25

Bacterial cells stored in sterile saline at 4°C retained their activity for at least 3 months. At this time, no aggregating property was present in the suspending fluid after removal of the bacterial cells.

To determine whether platelet aggregation was accompanied by the typical platelet release action, the loss of serotonin from serotonin-labeled platelets was measured. The plotted data (Fig. 2) revealed that approximately 60% of the original serotonin burden is released during platelet aggregation. Serotonin release to the extent of about 10% of the total is evident by 1 min, just before the sharp change in the aggregation curve. At the time when aggregation is 50% complete, serotonin discharge is approximately 85% complete.

Quantitative determinations of lactic dehydrogenase in PRP incubated with buffer, ADP, or an aggregating strain of *F. necrophorum* were virtually identical at values of 210 to 215 enzyme units per ml. Triton X-100 lysis of platelets released 1,500 to 1,800 U of lactic acid dehydrogenase depending upon the experiment. These data indicate that serotonin release is not the result of platelet dissolution.

Several known inhibitors of platelet aggregation were examined for their influence on the fusobacterial aggregation phenomenon (Table 1). Aspirin and quinacrine were selected because they are known inhibitors of the cyclooxygenase pathway of arachidonate metabolism (16, 29). At 6 min after the addition of *F. necrophorum* to uninhibited platelets, aggregation was always complete. Aspirin at 1.0 and 2.5 mM retarded the onset of aggregation slightly but its inhibitory effect was more clearly seen at 6 min when aggregation was only 25 and 12% complete, respectively. Quinacrine at 0.25 mM had no effect on the time of aggregation onset, although it was a distinct inhibitor of aggregation progress, permitting only 20% of the expected response at 6 min. EDTA and dipyrimadole, which may inhibit platelet aggregation by the same mechanism, were markedly inhibitory at 2.0 and 0.5 mM, respectively. Imipramine at 0.01 mM was slightly retardant and distinctly inhibitory.

GFP were not susceptible to aggregation by *F. necrophorum* in buffer (Fig. 3). Reconstitution with platelet-poor plasma allowed normal aggregation to occur. Reconstitution with fibrinogen also restored sensitivity to aggregation; however, a distinct lag was observed, extending to the 3-min mark, which was then followed by a rate of aggregation slower than that exhibited by GFP reconstituted with platelet-poor plasma.

When fusobacterial aggregating activity and biotype were compared, it was determined that none of the seven avirulent B biotype strains aggregated platelets. Cells of the single

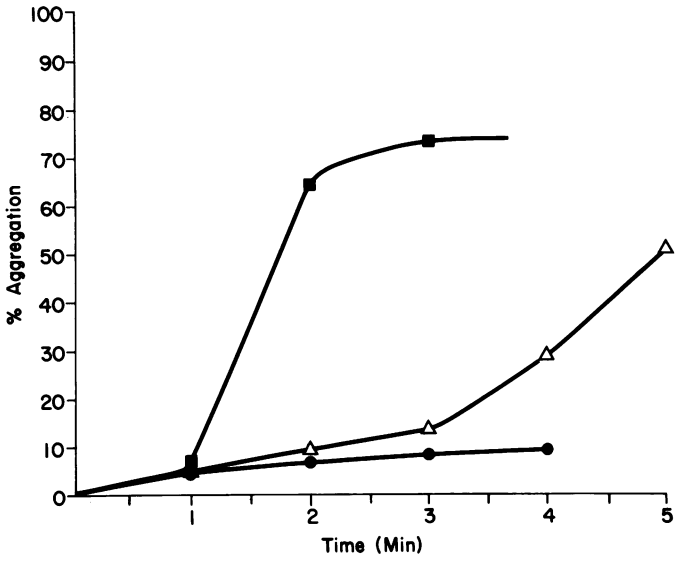

FIG. 3. The aggregative response of GFP to *F. necrophorum* 3080 is minimal in the absence of any additive (●) but is normal upon supplementation with platelet-poor plasma (■). The addition of fibrinogen (△) resulted in a delayed response to fusobacterium-induced aggregation.

mixed AB biotype tested were also inactive. Only strains of the virulent A type were capable of destabilizing platelets, 13 of the 16 strains were positive, and three strains were inactive within the time limit imposed.

DISCUSSION

The ability of the anaerobic *F. necrophorum* to effect platelet aggregation under aerobic conditions and after aerobic storage for as long as 3 months indicated that bacterial metabolism was not essential for this reaction. The failure of cell-free culture media or saline used to store washed cells to promote aggregation is evidence that an aggregating agent was not released from the bacteria and incriminates platelet-bacterial cell contact as an initial event leading to aggregation.

Platelet aggregation by *F. necrophorum* was similar to that seen with *Streptococcus pyogenes* (20) in several regards. Serotonin release paralleled aggregation without any delay. Aggregation continued into the serotonin release phase characteristic of platelet degranulation. The amount of serotonin released from prelabeled platelets never exceeded 70% of that initially imbibed by the cells. Release of serotonin from platelets was a nonlytic event, since the level of lactic acid dehydrogenase in the plasma-platelet-bacterial mixture remained constant during the release reaction. This is evidence that the complement system does not participate in the fusobacterium-platelet system even though these bacteria are known to have a potent lipopolysaccharide (27).

The inhibition of fusobacterium-stimulated platelet aggregation by EDTA emphasizes the known calcium dependence of the reaction and separates it from other cell-cell adhesive phenomena (38). Inhibition by dipyridamole is of interest because it interferes indirectly with the role of Ca^{2+} in platelet aggregation. Dipyridamole, by inhibiting phosphodiesterase, prevents the breakdown of cyclic AMP. Increases in cyclic AMP stimulate the removal of Ca^{2+} needed for aggregation (30). Based on inhibition by aspirin, the response of platelets to fusobacteria can be added to those systems associated with the cyclooxygenation of arachidonic acid. The ability of quinacrine, another cyclooxygenase inhibitor, to restrict platelet aggregation supports the data from the aspirin experiments. The additional inhibition of the platelet aggregation and release reaction by imipramine may be due to the action of this compound on membrane stability (23, 24).

To claim platelet aggregation as a virulence property, it would be optimal to demonstrate activity in all A-type strains. The failure to observe a platelet effect with 3 of these 16 strains may be explained by several means, e.g., loss of this capacity in culture or failure to observe for aggregating activity for a period longer than 5.5 min, the arbitrary time limit. Certainly the failure of any B or the AB biotype to activate platelets is interesting. The ability of pathogenic strains of *F. necrophorum* to protect themselves against host defense properties by creating fibrin-encapsulated abscesses could easily involve platelets. The importance of platelet aggregation and granule release in fibrinogen-fibrin conversion and other, perhaps only local, disturbances of the blood coagulation system is well known (33), and the data presented here show that virulent strains of *F. necrophorum* can activate the platelet system.

Septic thrombophlebitis with a gram-negative anaerobic bacterial etiology has been associated with human tonsillitis, pharyngitis, otitis media, septic abortion, and other conditions. These bacteria may be important also in fever of undetermined origin because of their propensity to produce septic thrombophlebitis (10). Biotyping of human isolates of *Fusobacterium necrophorum* by schemes appropriate to isolate from lower animals has not been successful (J. N. Berg, unpublished data), but determination of their virulence potential should now include their effect on blood platelets.

ACKNOWLEDGMENT

This investigation was supported by the University of Missouri-Columbia, School of Medicine, Research Council.

LITERATURE CITED

1. **Beachey, E. H., T. M. Chiang, I. Ofek, and A. H. Kang.** 1977. Interaction of lipoteichoic acid of group A streptococci with human platelets. Infect. Immun. **16**:649–654.
2. **Bergmeyer, H. U., E. Bernt, and B. Hess.** 1965. Lactic dehydrogenase, p. 736–741. *In* H. U. Bergmeyer (ed.), Methods of enzymatic analysis. Academic Press, Inc., New York.
3. **Bernheimer, A. W., and L. L. Schwartz.** 1965. Effects of staphylococcal and other bacterial toxins on platelets in vitro. J. Pathol. Bacteriol. **89**:209–223.
4. **Born, G. V. R., and M. J. Cross.** 1963. The aggregation of blood platelets. J. Physiol. **168**:178–195.
5. **Brecher, G., M. Schneiderman, and E. P. Cronkite.** 1953. The reproducibility and constancy of the platelet count. Am. J. Clin. Pathol. **23**:15–26.
6. **Czuprynski, C. J., and E. Balish.** 1981. Interaction of rat platelets with *Listeria monocytogenes*. Infect. Immun. **33**:103–108.
7. **DesPrez, R. M., S. Steckley, R. M. Stroud, and J. Hawiger.** 1980. Interaction of *Histoplasma capsulatum* with human platelets. J. Infect. Dis. **142**:32–39.
8. **Falker, W. A., Jr., and B. W. Burger.** 1981. Microbial surface interactions: reduction of the hemagglutination activity of the oral bacterium *Fusobacterium nucleatum* by absorption with *Streptococcus* and *Bacteroides*. Arch. Oral Biol. **26**:1015–1025.
9. **Fievez, L.** 1963. Etude comparee des souches de *Sphaerophorus necrophorus* isolees chez l'homme et l'animal. Presses Academiques Europeennes, Brussels.
10. **Finegold, S. M.** 1977. Anaerobic bacteria in human disease, p.

116, 368, and 471. Academic Press, Inc., New York.
11. **Gibbons, R. J., and J. vanHoute.** 1971. Selective bacterial adherence to oral epithelial surfaces and its role as an ecological determinant. Infect. Immun. **13**:661–666.
12. **Greenblatt, J., J. R. Boackle, and J. H. Schwab.** 1968. Activation of the alternate complement pathway by peptidoglycan from streptococcal cell wall. Infect. Immun. **19**:296–303.
13. **Hawiger, J., S. Steckley, D. Hammond, C. Cheng, S. Timmons, and R. M. DesPrez.** 1979. Staphylococci-induced human platelet injury mediated by protein A and IgG Fc fragment receptor. J. Clin. Invest. **64**:931–937.
14. **Hite, K. E., M. Locke, and H. C. Hesseltine.** 1949. Synergism in experimental infections with nonsporulating anaerobic bacteria. J. Infect. Dis. **84**:1–9.
15. **Hofstad, T.** 1984. Pathogenicity of anaerobic gram-negative rods: possible mechanisms. Rev. Infect. Dis. **6**:189–199.
16. **Holmsen, H.** 1976. Classification and possible mechanisms of action of some drugs that inhibit platelet aggregation. Ser. Haematol. **8**:50–80.
17. **Holmsen, H., and C. A. S. Dangelmaier.** 1977. Adenine nucleotide metabolism of blood platelets. X. Formaldehyde stops centrifugation-induced secretion after A23187-stimulation and causes breakdown of metabolic ATP. Biochim. Biophys. Acta **497**:46–61.
18. **Johnson, M. K., D. Boese-Marrazzo, and W. A. Pierce, Jr.** 1981. Effects of pneumolysin on human polymorphonuclear leukocytes and platelets. Infect. Immun. **34**:171–176.
19. **Kantor, F. S.** 1965. Fibrinogen precipitation by streptococcal M-protein. I. Identity of the reactants, and stoichiometry of the reaction. J. Exp. Med. **121**:849–859.
20. **Kurpiewski, G. E., L. J. Forrester, B. J. Campbell, and J. T. Barrett.** 1983. Platelet aggregation by *Streptococcus pyogenes*. Infect. Immun. **39**:704–708.
21. **Lages, B., M. C. Scrutton, and H. Holmsen.** 1975. Studies on gel-filtered human platelets: isolation and characterization in a medium containing no added Ca^{2+}, Mg^{2+}, or K^{+}. J. Lab. Clin. Med. **85**:811–825.
22. **Maisch, P. A., and R. A. Calderone.** 1981. Role of surface mannan in the adherence of *Candida albicans* to fibrin-platelet clots formed in vitro. Infect. Immun. **32**:92–97.
23. **Mills, D. C. B., I. A. Robb, and G. C. K. Roberts.** 1968. The release of nucleotides, 5-hydroxytryptamine and enzymes from human blood platelets during aggregation. J. Physiol. **195**:715–729.
24. **Mills, D. C. B., and G. C. K. Roberts.** 1967. Membrane active drugs and the aggregation of human platelets. Nature (London) **213**:35–38.
25. **Mongiello, J. R., and W. A. Falker, Jr.** 1979. Sugar inhibitions of oral *Fusobacterium nucleatum* hemagglutination and cell binding. Arch. Oral Biol. **24**:483–489.
26. **Morrison, D. C., L. F. Kline, Z. G. Oades, and P. M. Henson.** 1981. Mechanisms of lipopolysaccharide-initiated rabbit platelet responses: alternate complement pathway dependence of the lytic reaction. Infect. Immun. **20**:744–751.
27. **Nygren, H., G. Dahlen, and L. A. Nilsson.** 1979. Human complement activation by lipopolysaccharides from *Bacteroides oralis*, *Fusobacterium nucleatum*, and *Veillonella parvula*. Infect. Immun. **26**:391–396.
28. **O'Brien, J. R.** 1962. Platelet aggregation. Part II. Some results from a new method of study. J. Clin. Pathol. **15**:452–455.
29. **Ouyang, C., and C.-M. Teng.** 1979. The action mechanism of the purified platelet aggregation principle of *Trimeresurus mucrosquamatus* venom. Thromb. Haemostas. **41**:475–487.
30. **Rittenhouse-Simmons, S., and D. Deyken.** 1978. The activation by Ca^{2+} of platelet phospholipase A2. Effects of dibutyryl cyclic adenosine monosphosphate and 8-(N,N-diethylamino)-octyl-3,4,5-trimethoxybenzoate. Biochim. Biophys. Acta **543**: 409–422.
31. **Rotta, J., M. Ryc, K. Masek, and M. Zaoral.** 1979. Biological activity of synthetic subunits of streptococcus peptidoglycan. I. Pyrogenic and thrombocytolytic activity. Exp. Cell Biol. **47**:258–268.
32. **Rourke, F. J., S. S. Fan, and M. S. Wilder.** 1979. Anti-complementary activity of tuberculin: relationship to platelet aggregation and lytic response. Infect. Immun. **23**:160–167.
33. **Sanford, J. P., and J. P. Luby.** 1981. Infectious diseases, p. 6–8. *In* J. M. Dietschy (ed.), The science and practice of clinical medicine, vol. 8. Grune and Stratton, New York.
34. **Scanlan, C. M., J. N. Berg, and W. H. Fales.** 1982. Comparative *in vitro* leukotoxin production of three bovine strains of *Fusobacterium necrophorum*. Am. J. Vet. Res. **43**:1329–1333.
35. **Schmidt, K. H., and W. Kohler.** 1981. Interaction of streptococcal cell wall components with fibrinogen. I. Adsorption of fibrinogen by immobilized T-proteins of *Streptococcus pyogenes*. Immunobiology **158**:330–337.
36. **Sheth, N. K., V. P. Kurup, and B. A. Barron.** 1980. The role of *Aspergillus fumigatus* antigens in blood coagulation and platelet function. Microbios **28**:91–96.
37. **Skerl, K. G., R. A. Calderone, and T. Sreevalsan.** 1981. Platelet interactions with *Candida albicans*. Infect. Immun. **34**:938–943.
38. **Spaet, T. H., and I. Lejnieks.** 1969. A technique for estimation of platelet-collagen adhesion. Proc. Soc. Exp. Biol. Med. **132**:1038–1041.
39. **Steffen, E. K., and D. J. Hentges.** 1981. Hydrolytic enzymes of anaerobic bacteria isolated from human infections. J. Clin. Microbiol. **14**:153–156.